C. R. Chimie 17 (2014) 268–283

Contents lists available at ScienceDirect

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Bixin extraction from defatted annatto seeds

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ARTICLE INFO

Article history:
Received 8 August 2013
Accepted after revision 17 October 2013
Available online 13 January 2014

Keywords:
Annatto
Bixa orellana
Bixin
Pressurized liquid extraction
Low-pressure solvent extraction

ABSTRACT

Bixin is the major carotenoid in the seed of the Annatto plant (*Bixa orellana* L.). The aim of this study was to obtain extracts containing bixin from seeds that had been partially defatted by supercritical fluid extraction. Pressurized liquid extraction (PLE) and low-pressure solvent extraction (LPSE) methods were used, and the effects of the solvent, temperature, pressure, solvent mass to feed mass (S/F) ratio and ultrasonication were evaluated for the global yield (X_0(%)) and the bixin yield (BY(%)). Extraction conditions producing high yields of bixin were established for both the PLE and LPSE methods. Analysis of variance was used to examine the influence of the individual extraction variables in LPSE and PLE. For LPSE; significant effects were found for solvent, temperature, and the interactions of temperature with solvent and temperature with S/F. Solvent was the only variable that significantly affected X_0(%) and BY(%), for PLE. While ultrasonication did not significantly affect X_0(%) or BY(%), scanning electron microscopy analysis revealed structural changes in the vegetal matrix following this treatment.

1. Introduction

The use of additives to make food and other products more visually appealing is very common in the food and chemical industries, and so the interest in and demand for natural products have increased significantly in recent years. Due to certain requirements and consumer preferences for natural compounds, a global trend has arisen aiming to increase the use of natural products over synthetic versions. The growing demand for natural dyes is justified by their minimal or absent toxicity.

Annatto is a shrub native to the South American tropics, the natural reddish-yellow color of which is obtained from the outer coating of its seeds. The major pigments present are carotenoids, including a large amount of *cis*-bixin and other minor constituents, such as *trans*-bixin, *cis*-norbixin and *trans*-norbixin. Annatto is almost unique among the sources of carotenoids, as its pigment takes on a number of different chemical structures; the range of intense colors its compounds take include shades of red, orange and yellow. Annatto can be obtained from hydrophilic and hydrophobic extracts, and its pigments are very stable due to their interactions with protein compounds. Thus, it is an excellent candidate for a natural pigment to be used in cosmetics, pharmaceuticals and the food industry [1–3]. Unlike β-carotene, which is widely distributed in vegetables and fruit, bixin can only be found in annatto and comprises more than 80% of the total carotenoid content of its seeds [3].

Various techniques have been studied to develop clean extraction technologies with environmental benefits (so-called "green technologies"). Innovative technologies, such as ultrasound-assisted extraction [4–8], microwave-assisted extraction [9], supercritical fluid extraction [10–15] and accelerated solvent extraction [7,16,17], can be

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1631-0748/$ – see front matter
http://dx.doi.org/10.1016/j.crci.2013.10.010

combined to develop processes that are free of the residues of organic solvents. These techniques are used to extract bioactive substances to shorten the processing time, reduce solvent consumption, increase the extraction yield and improve the quality of the extracts. These studies are needed because of the general trend of the market to identify products that generate economic, social and environmental advantages [11,18]. Extraction with organic solvents is limited by the need for a solvent that is compatible with the end use of the product. In food, the dye will be subject to serious technical restrictions on the amount of residues from potentially toxic solvents [15].

Solid-liquid extraction or solvent extraction occurs through the selective dissolution of one or more solutes from a solid matrix by a liquid solvent. This unit operation is also called leaching, decoction, elution or low-pressure solvent extraction (LPSE). Regardless of the name, this technique is one of the most widely used operations in the chemical industry [18].

For instance, a certain liquid can be pressurized and heated at pressures and temperatures below its critical point and then employed in the extraction of several compounds, based on the potentially increased solubility of the compounds to be extracted and on the acceleration of the desorption kinetics of these compounds from the vegetable matrix. The liquid extraction process at pressures higher than ambient pressure and moderate to high temperatures has various names; in this paper, we use the name "pressurized liquid extraction" (PLE).

The extraction methods used for the production of dyes from annatto seeds may produce bixin or, by aqueous hydrolysis, the simultaneous extraction of norbixin [1,19].

The most commonly used methods to extract the pigments from annatto seeds are alkaline extraction (norbixin salt), extraction with oil (bixin) and extraction with solvents, such as ethyl acetate, ethanol, chloroform and acetone, to yield products with higher purity. These dyes differ in solubility and pigmentation [1].

The great demand for annatto extracts with high quality characteristics has accentuated the deficiencies of the commonly used processes to produce dyes. Typically, these techniques require high extraction times and provide a low efficiency, even while including the risk of the thermal degradation or oxidation of the pigment extracts, which requires the use of extraction techniques at milder conditions to avoid degradation.

Ultrasonic energy has been identified as an efficient tool to improve performance in different applications of analytical chemistry, such as the extraction of organic and inorganic compounds, homogenization, and dispersion of suspensions, among other applications [4–6,18]. The improvement of the extraction efficiency for organic compounds by the use of ultrasonication is based on the phenomenon of cavitation, which is produced in the solvent by the passage of sound waves [20–22]. In general, for ultrasound techniques to be efficient, an ultrasonic probe should be used, as described by Veggi et al. [4]; however, there have been reports in the literature of improvements in the processing performance even with ultrasonic bath-assisted extractions [8]. Nonetheless, it is import to observe that the extraction efficiency is a result of the solid matrix pretreatment, solvent, and ultrasound power.

The major part of bixin in annatto seeds is located in the outside layer of the seed. Nonetheless, there is a lipid layer strongly associated to the bixin in the seed; the removal of the lipidic layer during the extraction of bixin from annatto is the most difficult step. Bixin has a very low solubility in carbon dioxide while that of the lipids is very high. Therefore, we envisioned a process in two steps: (i) removal of the lipids using supercritical carbon dioxide followed (ii) by removal of bixin. The removal of the lipids using supercritical carbon dioxide has proven to be a very efficient process [11]. Additionally, as reported in literature [23] supercritical fluid extraction is an interesting tool for solid matrix pretreatment. Therefore, in this work, we are seeking for the best process to remove bixin from defatted annatto seeds. Therefore, two process were investigated PLE and LPSE. The effects of solvent, ratio of mass of solvent to mass of solvent, temperature, pressure and ultrasound assistance were evaluated on the total yields of extract and of bixin.

2. Material and methods

2.1. Material

Annatto seeds were partially defatted as described by Albuquerque [11]. Briefly, annatto seeds of the Piave variety were defatted at 313 K and 20 MPa using supercritical CO_2. The extraction was carried out in a commercial SFE unit (Thar Technologies, SFE-2 × 5LF-2-FMC, Pittsburgh, Pennsylvania, USA).

2.2. Characterization of the raw materials

The real density (ρr) of the seeds was determined following the method of helium pycnometry using a gaseous pycnometer (Quantachrome, Ultrapyc™ 1200e, Boynton Beach, Florida, USA) in the Analytical Central/Institute of Chemistry/Unicamp. Apparent density (ρ_a) was calculated as the ratio of the mass of raw material used to fill the extraction cell to its volume. The porosity of the bed and the particles (ε) was determined as $(1-(\rho_a/\rho_r))$. The average diameter (dp) of the seeds was determined according to the FAO/WHO report [1], using the geometric means of the height, width and thickness of twenty randomly selected seeds, as measured by a universal pachymeter. For the analysis of their chemical composition, the samples were milled (Tecnal, model TE-631, Piracicaba, São Paulo, Brazil). The moisture [24], ash [25], lipid [26] and protein [27] contents were determined. Scanning electron microscopy analysis was performed at the Analytical Laboratory of Resources and Calibration (LRAC) at the School of Chemical Engineering (FEQ)/UNICAMP, São Paulo, Brazil. The aim was to analyze the seed surface, evaluating the effects of the extraction conditions used in this study on the structure of the pericarps of the seeds, where bixin is most commonly located. A coating of gold with a thickness of 92 Å was applied by metallic sputter coating (Polaron SC7620 sputter coater, VG Microtech, Uckfield, UK) in the presence of an inert gas, such as argon. To obtain the micrographs, a